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Iridoid Glucosides from *Lamium garganicum* subsp. *laevigatum*

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Phytochemical investigations on the above-ground parts of *Lamium garganicum* subsp. *laevigatum* resulted in the isolation of 7 iridoid glucosides: shanzhiside methyl ester (**1**), barlerin (8-*O*-acetylshanzhiside methyl ester) (**2**), 6-*O*-syringyl-8-*O*-acetylshanzhiside methyl ester (**3**), 6β-hydroxyipolamiide (**4**), lamalbide (**5**), dehydropenstemoside (**6**), and sesamoside (**7**). The structure of the iridoids was elucidated by spectroscopic (UV, IR, 1D- and 2D-NMR, and ESI-MS) evidence.

Key Words: *Lamium garganicum* L. subsp. *laevigatum*, Lamiaceae, iridoid glucosides.

Introduction

The genus *Lamium* L. (Lamiaceae) comprises about 40 species distributed in Europe, Asia, and Africa.[1] There are 30 *Lamium* species recorded in the Flora of Turkey.[2] Some *Lamium* species have been used in folk medicine. *Lamium album* is the most popular species, with blood tonic, uterotonic, astringent, antispasmodic, and anti-inflammatory activities. *L. maculatum* has been used in Chinese folk medicine for treatment of trauma, fracture, injuries from falls, putrescence, paralysis, leukorrhea, and hypertension.[3,4] *Lamium album* and *L. maculatum* have been used in Anatolian folk medicine as tonics.[5] In previous studies, different types of compounds, such as iridoid and secoiridoid glucosides,[6–12] phenylpropanoids,[4,11,14] flavonoids,[4,13,14] essential oils,[15] phytoecdysteroids,[11,16] benzoxazinoids,[11] and triterpene saponins,[17] as well

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as megastigmenone[18] and hemiterpene[19] type compounds, have been isolated from *Lamium* species. To date, shanzhiside methyl ester, 8-*O*-acetylshanzhiside methyl ester (= barlerin), lamalbide, and sesamoside have been identified in *L. garganicum*[10,11] and the essential oil from *L. garganicum* subsp. *laevigatum* has been examined by GC and GC-MS.[15] As a part of our ongoing phytochemical studies of members of the Lamiaceae growing in Turkey, we investigated *Lamium garganicum* L. subsp. *laevigatum* Arcangeli. The current study describes the isolation and structure elucidation of the iridoid glucosides from the title plant.

Experimental

General Experimental Procedures: Optical rotations were determined on a Rudolph Autopol IV Automatic polarimeter using MeOH as solvent. UV spectra were recorded in MeOH using an Agilent 8453 spectrophotometer. 1D- and 2D-NMR measurements were recorded at room temperature on a Bruker AVANCE 400 MHz spectrometer for **1**, **3**-**5** and **7** and on a Varian Unity Inova 500 MHz spectrometer for **2** and **6** (^{1}H: 500 and ^{13}C: 125 MHz). All compounds were dissolved in CD_3OD and the spectra were referenced according to the solvent peaks (δ_H 3.31 or δ_C 49.0). ESI-MS was performed on a Water ZQ Mass Spectrometer (ESI positive ion, MeOH, m/z). Polyamide 6 (Fluka, 50-160 μm) and Kieselgel 60 (Merck, 0.063-0.200 mm) were used for open column chromatography (CC). Medium-pressure liquid chromatographic (MPLC) separations were carried out on Büchi (3 x 25 cm) glass columns packed with LiChroprep C_{18} (Merck, 40-63 μm), using a Büchi 681 pump. LiChroprep C_{18} (Merck, 40-63 μm) was used for vacuum-liquid chromatography (VLC) (column 5.2 x 10 cm, vacuum by H_2O aspiration). TLC analyses were carried out on pre-coated Kieselgel 60 F_{254} aluminum plates (Merck). Compounds were detected by UV fluorescence and spraying 1% vanillin/H_2SO_4, followed by heating at 100 °C for 1-2 min.

Plant Material: *Lamium garganicum* L. subsp. *laevigatum* Arcangeli (Lamiaceae) was collected in July 2005 at Uludağ (Bursa), in the vicinity of the Kuşaklıkaya region. Voucher specimens (HUEF-05004) have been deposited at the Herbarium of the Faculty of Pharmacy, Hacettepe University, Ankara, Turkey.

Extraction and Isolation: Dried and powdered aerial parts of *L. garganicum* subsp. *laevigatum* (285 g) was extracted with MeOH (4 x 1.5 L) at 40 °C and combined MeOH extracts were concentrated under reduced pressure. The resultant extract was then dissolved in H_2O (150 mL) and the water-soluble portion was partitioned between CH_2Cl_2 (4 x 150 mL) and *n*-BuOH (4 x 150 mL). An aliquot (20 g) of the n-BuOH extract was chromatographed over polyamide (100 g), eluting with H_2O, followed by increasing concentrations of MeOH in H_2O (25%, 50%, 75%, and 100%, each 250 mL) to yield 11 main fractions (Frs. A-K). Fr. A (10 g) was subjected to VLC on LiChroprep C_{18} and elution with H_2O, followed by MeOH-H_2O (0%→100%) solvent system, yielded 9 fractions (Frs. A_1-A_9). Fr. A_5 (505 mg) was first subjected to silica gel column chromatography with a gradient of EtOAc-MeOH-H_2O (10:5:2→100:17:13) to give 5 subfractions (Frs. A_{5A}-A_{5E}). Fr. A_{5A} was found to be compound **7** (40.5 mg) in pure form. Fr. A_{5D} (36 mg) was subjected to VLC (LiChroprep C_{18}) and elution with MeOH-H_2O (0%→100%) mixture afforded compound **4** (10.8 mg). VLC fractionation of fr. A_{5E} (45 mg) on LiChroprep C_{18} using the same solvent system yielded compound **5** (2 mg). Fr. A_6 (1.2 g) was first applied to silica gel CC with EtOAc-MeOH-H_2O (10:5:2→100:30:2) mixture and a fraction rich in compound **1** (Fr. A_{6B}, 454 mg) was collected. Fr. A_{6B} was then applied to MPLC (LiChroprep C_{18}) and eluted with H_2O followed by increasing concentrations of MeOH in H_2O (0%→100%) to give compound **1** (98 mg). CC fractionation of fr. A_8 (295 mg) on silica

gel with EtOAc-MeOH-H_2O (10:5:2→100:17:13) mixture as eluent gave compound **6** (20 mg). Fr. A_9 was compound **2** (67 mg) in pure form. Fractionation of fr. D (390 mg) by MPLC (LiChroprep C_{18}; MeOH-H_2O (0%→100%)) afforded 3 subfractions (Frs. D_1-D_3). Fr. D_2 (115 mg) was then purified on a silica gel column, eluting with EtOAc-MeOH-H_2O (10:10:0→100:17:13) mixture, and yielded compound **3** (80 mg).

Shanzhiside methyl ester (1): $C_{17}H_{26}O_{11}$; $[\alpha]_D^{33}$ -122° (*c* 0.1, MeOH); UVgλ_{max} (MeOH) nm: 236; IR υ_{max} (KBr) cm^{-1} 3408, 2929, 1690, 1645, 1299, 1078; ^{13}C (100 MHz, CD_3OD) and ^1H (400 MHz, CD_3OD) NMR data are given in Table 1; ESI-MS: m/z 429 $[M+Na]^+$.

Table 1. ^{13}C-NMR and ^1H-NMR spectroscopic data for compounds 1-3 (in CD_3OD)[a].

		1			2			3		
C/H	**DEPT**	**δ_C**	**δ_H**	**J (Hz)**	**δ_C**	**δ_H**	**J (Hz)**	**δ_C**	**δ_H**	**J (Hz)**
1	CH	94.8	5.57 d	(2.4)	95.7	5.92 d	(2.4)	95.4	5.91 d	(3.4)
3	CH	152.8	7.41 s		153.7	7.46 d	(1.4)	154.5	7.55 s	
4	C	111.5			109.8			108.7		
5	CH	41.5	3.00 dd	(10.1/3.2)	42.3	3.08 dd	(8.9/1.4)	40.1	3.46 d	(8.5)
6	CH	77.5	4.06 ddd	(6.4/6.0/3.2)	76.0	4.34 m		79.6	5.49 br.d	(5.2)
7	CH_2	49.2	2.01 dd	(13.2/6.4)	47.7	2.22 d	(14.9)	45.1	2.50 d	(15.6)
			1.83 dd	(13.2/6.0)		2.06 dd	(14.9/5.4)		2.23 dd	(15.6/5.2)
8	C	79.1			89.8			89.2		
9	CH	51.8	2.62 dd	(10.1/2.4)	50.0	3.01 dd	(8.9/2.2)	50.5	3.09 dd	(8.5/3.4)
10	CH_3	24.7	1.26 s		22.3	1.53 s		21.8	1.61 s	
11	C	169.7			168.3			168.5		
COO$\underline{C}H_3$	CH_3	51.9	3.73 s		51.8	3.74 s		51.9	3.69 s	
O$\underline{C}OCH_3$	C				173.1			172.7		
OCO$\underline{C}H_3$	CH_3				22.3	2.03 s		22.3	1.91 s	
1'	CH	99.8	4.63 d	(8.0)	100.4	4.65 d	(7.9)	100.3	4.71 d	(7.8)
2'	CH	74.7	3.17 dd	(8.8/8.0)	74.7	3.19 dd	(8.8/7.9)	74.7	3.17 dd	(7.8/8.0)
3'	CH	78.4	3.36 t	(8.8)	78.1	3.38 t	(8.8)	78.0	3.36 t	(8.9)
4'	CH	71.7	3.26 dd	(8.8/8.7)	71.7	3.29 dd	(8.6/8.8)	71.7	3.23 dd	(8.4/8.5)
5'	CH	78.1	3.32 m		78.4	3.31 m		78.5	3.32 m	
6'	CH_2	62.9	3.89 dd	(11.9/2.2)	63.0	3.93 dd	(12.0/2.1)	63.0	3.90 dd	(11.9/2.2)
			3.65 dd	(11.9/6.0)		3.67 dd	(12.0/6.1)		3.67 dd	(11.9/5.6)
1''	C							121.7		
2''	CH							108.5	7.35 s	
3''	C							149.0		
4''	C							142.4		
5''	C							149.0		
6''	CH							108.5	7.35 s	
C=O	C							167.4		
Ar. OCH_3	CH_3							57.1	3.89 s	

[a] ^{13}C: 100 MHz; ^1H: 400 MHz for **1** and **3**; ^{13}C: 125 MHz; ^1H: 500 MHz for **2**

Barlerin (2): $C_{19}H_{28}O_{12}$; $[\alpha]_D^{33}$ -79° (*c* 0.1, MeOH); UVgλ_{max} (MeOH) nm: 234; IR υ_{max} (KBr) cm^{-1} 3400, 2930, 1700, 1633, 1286, 1092; ^{13}C (125 MHz, CD_3OD) and ^1H (500 MHz, CD_3OD) NMR data are given in Table 1; ESI-MS: m/z 471 $[M+Na]^+$.

6-*O*-Syringyl-8-*O*-acetylshanzhiside methyl ester (3): $C_{28}H_{36}O_{16}$; $[\alpha]_D^{33}$ -62° (*c* 0.1, MeOH); UVgλ_{max} (MeOH) nm: 278, 214; IR υ_{max} (KBr) cm^{-1} 3446, 2940, 1713, 1637, 1372, 1282, 1080; ^{13}C (100 MHz, CD_3OD) and ^1H (400 MHz, CD_3OD) NMR data are given in Table 1; ESI-MS: m/z 651 $[M+Na]^+$.

6β-hydroxyipolamiide (4): $C_{17}H_{26}O_{12}$; $[\alpha]_D^{33}$ –161° (*c* 0.2, MeOH); UVgλ_{max} (MeOH) nm: 231; IR υ_{max} (KBr) cm^{-1} 3400, 2928, 1710, 1645, 1395, 1310, 1080; ^{13}C (100 MHz, CD_3OD) and ^1H (400 MHz, CD_3OD) NMR data are given in Table 2; ESI-MS: m/z 445 $[M+Na]^+$.

Table 2. ^{13}C-NMR and ^{1}H-NMR spectroscopic data for compounds 4-7 (in CD_3OD)[a].

	4				**5**				**6**				**7**			
	DEPT	**δ_C**	**δ_H**	**J (Hz)**	**DEPT**	**δ_C**	**δ_H**	**J (Hz)**	**DEPT**	**δ_C**	**δ_H**	**J (Hz)**	**DEPT**	**δ_C**	**δ_H**	**J (Hz)**
1	CH	93.9	5.85 brs		CH	94.9	5.60 d	(1.8)	CH	94.9	5.85 d	(2.4)	CH	96.9	5.49 d	(8.4)
3	CH	154.1	7.50 s		CH	152.8	7.40 s		CH	155.1	7.52 s		CH	155.5	7.58 s	
4	C	114.4			C	111.7			C	112.8			C	113.1		
5	C	70.3			CH	37.7	2.92 dd	(10.8/ 3.4)	C	73.7			C	74.9		
6	CH	74.6	4.06 dd	(8.8/ 6.4)	CH	78.4	3.94 dd	(4.4/ 3.4)	CH	78.9	4.53 d	(1.1)	CH	77.6	4.32 brs	
7	CH_2	48.2	2.01 dd	(12.4/ 8.8)	CH	79.1	3.53 d	(4.4)	CH	128.9	5.55 dd	(2.4/ 1.6)	CH	66.0	3.47 brs	(8.8)
			1.90 dd	(12.4/ 6.4)												
8	C	75.5			C	78.6			C	143.5			C	63.0		
9	CH	60.7	2.60 s		CH	49.3	2.79 d	(10.8)	CH	56.8	3.13 d	(2.4)	CH	54.5	2.52 d	
10	CH_3	23.9	1.14 s		CH_3	22.1	1.20 s		CH_3	15.8	1.83 d	(1.2)	CH_3	17.8	1.51 s	
11	C	168.3			C	169.6			C	168.3			C	169.1		
COO$\underline{C}$H$_3$	CH_3	51.9	3.74s		CH_3	51.9	3.72 s		CH_3	51.8	3.74 s		CH_3	51.9	3.75 s	
1'	CH	99.8	4.59 d	(7.9)	CH	99.8	4.60 d	(7.9)	CH	100.0	4.59 d	(7.9)	CH	99.9	4.73 d	(8.0)
2'	CH	74.4	3.21 dd	(8.6/ 7.9)	CH	74.7	3.23 dd	(9.0/ 7.9)	CH	74.4	3.23 dd	(8.8/ 7.9)	CH	74.8	3.23 dd	(9.2/ 8.0)
3'	CH	77.4	3.39 t	(9.2)	CH	77.9	3.38 t	(8.7)	CH	77.5	3.38 t	(8.8)	CH	77.8	3.38 t	(9.2)
4'	CH	71.8	3.29 t	(8.6)	CH	71.7	3.29 t	(8.7)	CH	71.7	3.29 t	(8.6)	CH	71.8	3.26 t	(8.4)
5'	CH	78.5	3.32 m		CH	78.1	3.31 m		CH	78.5	3.31 m		CH	78.8	3.32 m	
6'	CH_2	62.9	3.90 dd	(11.9/ 2.0)	CH_2	62.8	3.88 dd	(11.9/ 2.1)	CH_2	62.8	3.93 dd	(12.0/ 2.1)	CH_2	63.1	3.93 dd	(11.9/ 2.1)
			3.67 dd	(11.9/ 5.9)			3.65 dd	(11.9/ 5.9)			3.67 dd	(12.0/ 6.1)			3.62 dd	(11.9/ 5.9)

[a] ^{13}C: 100 MHz, ^{1}H: 400 MHz for **4**,**5** and **7**; ^{13}C: 125 MHz , ^{1}H: 500 MHz for **6**

Lamalbide (5): $C_{17}H_{26}O_{12}$; $[\alpha]_D^{33}$ -123° (*c* 0.1, MeOH); UVgλ_{max} (MeOH) nm: 235; IR υ_{max} (KBr) cm^{-1} 3408, 2930, 1727, 1697, 1642, 1290, 1074; ^{13}C (100 MHz, CD_3OD) and ^1H (400 MHz, CD_3OD) NMR data are given in Table 2; ESI-MS: m/z 445 $[M+Na]^+$.

Dehydropenstemoside (6): $C_{17}H_{24}O_{11}$; $[\alpha]_D^{33}$ -94° (*c* 0.1, MeOH); UVgλ_{max} (MeOH) nm: 234, 204; IR υ_{max} (KBr) cm^{-1} 3384, 2920, 1698, 1631, 1294, 1076; ^{13}C (125 MHz, CD_3OD) and ^1H (400 MHz, CD_3OD) NMR data are given in Table 2; ESI-MS: m/z 427 $[M+Na]^+$.

Sesamoside (7): $C_{17}H_{24}O_{12}$; $[\alpha]_D^{33}$ -77° (*c* 0.1, MeOH); UVgλ_{max} (MeOH) nm: 235; IR υ_{max} (KBr) cm^{-1} 3410, 2925, 1696, 1635, 1304, 1043; ^{13}C (100 MHz, CD_3OD) and ^1H (400 MHz, CD_3OD) NMR data are given in Table 2; ESI-MS: m/z 443 $[M+Na]^+$.

Results and Discussion

From the aerial parts of *L. garganicum* subsp. *laevigatum* 7 iridoid glucosides (**1-7**) were isolated by fractionation of the *n*-BuOH extract prepared from the overground parts of the title plant through a column chromatography on polyamide, followed by VLC or MPLC on LiChroprep C_{18} and open column chromatography on silica gel (Figure).

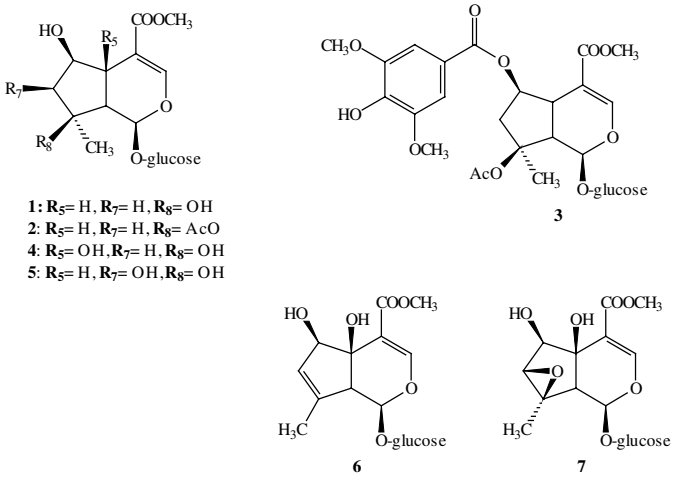

Figure. Iridoid compounds (**1-7**) isolated from *Lamium garganicum* subsp. *laevigatum.*

The UV spectra for compounds **1**, **2**, **4-7** revealed their λ_{max} at 231-236 nm, indicative of an α,β-unsaturated carbonyl moiety within each of the molecules. However, the UV spectrum of **3** (λ_{max} 278, 214 nm) showed the presence of an additional aromatic ring in the C-4 substituted iridoid skeleton. Their IR spectra displayed absorption bands typical for hydroxyls, a carbonyl function and an enolic double bond. The ^1H-NMR spectra of compounds **1-7** (Tables 1 and 2) showed proton signals corresponding to an enol-ether system conjugated with a carbomethoxyl group (δ_H 7.40-7.58, H-3; δ_H 3.69-3.75, COO*Me*), an oxymethine (δ_H 3.94-5.49, H-6), and a β-glucose moiety (δ_H 4.59-4.73, H-1'). In addition to these signals, a tertiary methyl (δ_H 1.14-1.61, H_3-10) signal was observed for compounds **1-5** and **7**, while a deshielded methyl signal appeared in the ^1H-NMR spectrum of compound **6** (δ_H 1.83). The secondary hydroxyl function at C-6 in all compounds (**1-7**) was assigned as β by comparing the ^{13}C-NMR data of the compounds with those of model C-6 (OH) epimeric iridoids.[20,21] The complete assignments of all proton and carbon resonances

were based on the DEPT, DQF-COSY, HSQC, HMBC, and NOESY experiments. Compounds **1**, **2**, **5** and **7** were identified as shanzhiside methyl ester (**1**),[21] barlerin (8-*O*-acetylshanzhiside methyl ester (**2**),[21] lamalbide (**5**),[22] and sesamoside (**7**),[21] respectively, by direct comparison of their spectroscopic data with those published in the literature.

The molecular formula of compound **3** was determined to be $C_{28}H_{36}O_{16}$ on the basis of positive ion ESI-MS (m/z 651 $[M+Na]^+$). ^{13}C-NMR and ^{1}H-NMR data of **3** (Table 1) were identical to those of barlerin (**2**) with additional proton (δ_H 7.35, s, 2H, H-2"/6" and 3.89, s, 6H, Ar.-OCH_3) and carbon (δ_C 167.4, C=O; and 108.5, C-2"/6") resonances, which could be assigned to a syringyl moiety. A 180 mass unit difference between compounds **3** and **2** also supported the presence of a syringyl moiety within **3**. Downfield chemical shifts of both C-6 (δ_C 79.6) and H-6 (δ_H 5.49) resonances, compared with those of **1** and **2**, showed that the syringyl moiety occupied the C-6 position. This proposal was also confirmed by the HMBC couplings observed from H-5 (δ_H 3.46) and H_2-7 (δ_H 2.50 and 2.23) to C-6. Therefore, on the basis of the above evidence and the optical rotation value, the structure of compound **3** was determined as 6-*O*-syringyl-8-*O*-acetylshanzhiside methyl ester.[21]

The positive-ion ESI-MS of compound **4** exhibited a molecular ion peak $[M+Na]^+$ at m/z 445, consistent with the molecular formula $C_{17}H_{26}O_{12}$. The ^{13}C-NMR and ^{1}H-NMR data of **4** (Table 2) were almost identical to those of **1**. However, the splitting pattern of the proton resonances of H-3 (δ_H 7.50, s) and H-9 (δ_H 2.60, s) suggested that C-5 is substituted. A 16 mass unit difference between **4** and **1** suggested that presence of a hydroxyl group occupying the C-5 position. Furthermore, prominent NOE correlations between H-1/H_3-10 and H-6/H_3-10 indicated that these protons are on the same side (α) of the cyclopentane pyrane ring system. Thus, the structure of compound **4** was assigned as 6β-hydroxyipolamiide and the optical rotation value of **4** supported this assumption.[21]

The ESI-MS of **6** revealed the $[M+Na]^+$ ion at m/z 427 in the positive ion mode, which indicated a molecular mass of 404 Da, compatible with the molecular formula $C_{17}H_{24}O_{11}$. In the ^{13}C-NMR and ^{1}H-NMR data of **6** (Table 2), there were signals arising from an olefinic methine (C-7, δ_H 5.55 and δ_C 128.9), an olefinic quaternary carbon (C-8, δ_C 143.5) atom, and a deshielded methyl doublet (C-10, δ_H 1.83, d, J= 1.2 Hz). H_3-10 protons showed HMBC correlations to C-7, C-9 (δ_C 56.8), and C-8, and H-7 displayed heteromultiple long-range correlations to C-5 (δ_C 73.7), C-6 (δ_C 78.9), C-9 (δ_C 56.8), and C-10 (δ_C 15.8), confirming the position of the double bond at C-7. Thus, on the basis of its NMR data and optical rotation value, compound **6** was identified as dehydropenstemoside.[23]

The cyclopentanopyran ring system of the above iridoid glycosides was supposed to be universallygβsβ-*cis*-fused and therefore the stereochemistry was determined by the interpretation of 1D- and 2D-NMR data and by analogy with the iridoids of known stereochemistry. Nevertheless, reports on the occurrence of iridoids with *trans*-fused ring junctions in plants[24−28] have opened up a new field of discussion on the determination of the relative stereochemistry of iridoids. The relative stereochemistry of these *trans*-fused iridoids was mainly determined based solely on the detailed analysis of the ^{1}H-^{1}H NOESY experiments; however, in some cases it was proved by X-ray crystallography.[24] In the present work, the ^{1}H-NMR and ^{13}C-NMR data of the isolated barlerin (**2**) and 7,8-didehydropenstemoside (**6**) were almost identical to those of 2 compounds presumed to be 6,9-*epi*-8-*O*-acetylshanzhiside methyl ester and 5,9-*epi*-7,8-didehydropenstemoside, previously isolated from *Eremostachys glabra*.[28] Moreover, compounds **2** and **6** both exhibited the unusual NOE interactions between H-1/H-9. Therefore, **2** and **6** could also be assumed to belong to the 9-epimeric iridoids. However, Franzyk

et al.[29] have stated that the structure of 10-hydroxy-8-*epi*-hastatoside (10α-CH_2) previously isolated from *Penstemon secundiflorus* and initially assigned due to the NOE interaction between H-1/H_2-10 should be revised to 10-hydroxyhastatoside (10β-CH_2), expressing that the NOE interaction between 2 noncontiguous protons on different faces of an iridoid skeleton is possible. In another work, Taşdemir et al.[30] also detected some unusual NOE interactions between H-1/H-9, H-1/H_3-10 and H-5/H-6 for the iridoid glucosides 6-*O*-acetylajugol and 7,8-epoxy-8-*epi*-loganic acid isolated from *Leonurus persicus*. However, performing molecular modeling and X-ray experiments it was concluded that in some conformations H-1(α) and H-9(β) protons as well as H-5(β) and H-6(α) protons of an iridoid cyclopentane ring were getting close in space and therefore exhibited the unexpected NOEs. As a consequence of these results, the structures of 6,9-*epi*-8-*O*-acetylshanzhiside methyl ester and 5,9-*epi*-7,8-didehydropenstemoside have recently been revised to **2** and **6**, respectively.[31] Thus, despite the presence of NOE interaction between H-1/H-9 in the NOESY spectra of **2** and **6** we assigned the β configuration to H-9 for both iridoids on the basis of the above-mentioned experimental results. The β-orientations of the C-6(OH) group in **2** and **6** were also assigned by comparing their NMR data with those published for related iridoids.[21] The relative configurations of the chiral centers of the remaining isolates (**1**, **3**-**5**, **7**) were established in the same manner. Finally, the optical rotation values of the isolates also supported the proposed structures for **1**-**7**.

Alipieva et al.[11] isolated shanzhiside methyl ester (**1**), 8-*O*-acetyl shanzhiside methyl ester (= barlerin) (**2**), lamalbide (**5**), and sesamoside (**7**) from a Bulgarian population of *Lamium garganicum* and employed the iridoid glucosides as chemotaxonomic markers of the genus *Lamium*. The main compounds found in the present investigation are all C_{10} iridoids with 11-$COOCH_3$ substitution, which is in good agreement with the Bulgarian results cited above. Using DNA sequencing results, Wink and Kaufmann[32] have shown that the genus *Lamium* is separated into 2 clades, where *L. garganicum* is a member of a clade accumulating 11-COOR iridoids. Thus, the iridoid composition of *L. garganicum* subsp. *laevigatum* is in good agreement with this classification. This is the first report of 6-*O*-syringyl-8-*O*-acetylshanzhiside methyl ester (**3**), 6β-hydroxyipolamiide (**4**), and dehydropenstemoside (**6**) in the genus *Lamium*.

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